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Turgidity in Mycelia

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Although the question of the mechanics of growth has been canvassed since the time of Nägeli by so many writers as Schmitz, Krabbe, Wortmann, Strasburger, Eschenhagen, Askenacy, Zacharias, Noll, Schwandener, Strange, Reinhardt, Hegler, Pfeffer, True, and Copeland, we must still agree with Krabbe, that an acceptable theory of growth does not exist. Especially are we indebted to the researches of Pfeffer as set forth in the "Druck und Arbeitsleistung durch wachsende Pflanzen" and the second part of "Studien zur Energetik der Pflanzen" for an elucidation of the various phenomena associated with growth. Especial attention is called by Pfeffer to the fact that the mechanics of growth may not only vary in different cases, but that all growth is dependent upon the coöperation of many factors. Consequently the retarding or quickening of the rate of growth may not be ascribed to any one agency. So in regard to turgidity its action may be more than counter-balanced by other forces, and we find no "fixed relation between the turgor force, stretching of membrane, and rate of growth." The present work was undertaken to determine just what relation turgidity sustains to growth when all other conditions, so far as possible, are kept constant and only known variants are introduced. The hyphae of fungi were used for these observations. They were grown in nutrient solutions of varying degrees of concentration and transferred to solutions of higher or lower concentration. In this way wide variation of turgidity was created, and as the moment of the renewal of growth in the hyphae

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could easily be detected, it was possible to test the turgor force at the instant of the recovery from the effect of the change of substratum. The basis of all media used in the experiments which are here recorded was a nourishing solution composed of 3% cane sugar, .5% peptone and .5% beef extract, and from this were made solutions containing various gram-molecule percentages of potassium nitrate. By this means the plants were always afforded a constant nourishment and the effects of the introduction of a known factor, potassium nitrate, could readily be determined. In many respects the hyphae of the lower fungi are especially adapted to this character of work since they are easily cultivated and handled in microscopic examinations, and possess, to a marvelous degree, adaptability to varying degrees of concentration without injurious results, and are so simple in structure that changes of culture media produce immediate reactions. Many will grow in a saturated potassium nitrate solution, about 23%, and some will endure a transfer from relatively strong to zero solutions without bursting. The suitableness of these plants for measuring the turgor force is more apparent when we consider that Pfeffer has determined that in an artificial cell a 1% potassium nitrate solution produced a pressure 175.8 cm. of mercury, and that this estimate is low since the precipitation membrane is somewhat permeable for potassium nitrate.

It was found impossible to study the behavior of the mycelia in gelatine or agar since these media not only dissolve at relatively low concentrations of the substratum, but they are also objectionable as being somewhat unstable and permitting a rather slow and gradual penetration of the various culture solutions. Gypsum proved a fairly satisfactory means of fixing the spores for germination and subsequent study. The powdered gypsum was mixed into a paste with water, and to this mass the spores were added. The mass was then readily pressed out into a thin plate between glass slides, and hardens in a few minutes. These thin plates, however, were somewhat difficult to fasten to the slides, and are liable to break during an experiment. So attempts were made to germinate the spores in floss silk and strands of cotton fibers fastened to the cover glass with drops of balsam, and this led to a very simple and practical culture method. The spores were fastened to the

cover glass with a film of dilute gelatine and covered with a narrow strip of silk paper or any thin unsized paper about 5 mm. long and 1 mm. wide. Each end of this paper had been previously touched with melted shellac so that a drop adhered to it. By applying a hot rod over the drop of shellac it is melted, fastening the paper firmly to the cover glass. In this way the paper is spread out smoothly over the spores, holding them firmly on the glass. The spores will germinate and push out from under the strip of paper and the mount can be handled without fear of destroying the hyphae, readily changed from one fluid to another and the growth studied in a hanging drop with ease. Furthermore the same hyphae can be observed during the entire experiment, and any error that might arise from a change from one filament to another can be avoided. Every precaution was taken to secure uniform external conditions. Repeated tests during the day showed that the ordinary variations of temperature produced no effect on the turgor force and rarely was there a variation of over one and a-half degrees during the majority of the experiments. The work prosecuted in the summer, however, was subject to a much more considerable range of variation than in the winter.

In regard to illumination, the alternation of light and darkness and different degrees of light intensity gave no different results from those obtained when the plants were grown in the dark room and received illumination only from the mirror of the microscope for a few moments at the time of the observations. For the purpose of uniformity all the cultures and examinations were made under the same conditions so far as possible and the greater part of the experiments were conducted under exceptionally uniform conditions.

The spores used were always taken from recent cultures since they produced plants of more uniform vigor. But even with this precaution here was presented the most serious difficulty in the work. For, having sown a few spores on a cover glass, not only do some fail to germinate but some are very slow to grow and others show a great vigor. Again, some will produce almost from the start a branched series of filaments and others will develop lateral branchlets only after extended growth. Now it is evident that

these variations are the expression of different conditions and forces existing in the plant and if the measurement of the turgidity is to be of any value it must be determined from plants presenting a uniform vigor of growth. It was often a difficult task to find in successive cultures hyphae showing the same vigor and condition of growth. The fluctuations in the rate of growth due in some cases to preparations for branching on the part of the plant and in other instances due to no apparent cause, rendered it difficult to select plants in the various experiments that would give real comparative values. This difficulty presented itself again when changes from one media to another had been made. For perhaps the hyphae under observation would not respond to the change for half an hour or longer after others had showed the desired effects. The question then presents itself, How much weight can be given to any measurement in such an instance? Obviously such conditions necessitated repetition of experiments many times to arrive at any conclusions.

To secure the most uniform conditions possible the spores, prepared as above described, were germinated in closed Stender dishes, 8 × 4½ cm., the cover glasses being just covered with the fluid. By this means the spores and germinating plants were exposed to the same atmospheric conditions and always had the same volume of nutrient solution to draw upon, while the solution could not materially absorb or evaporate water—factors of vital importance to the success of the work. By removing the covers of the dishes the cultures could readily be examined on the table of the microscope without danger of disturbing them and when found ready for use the cover glass was removed, carefully freed of the fluid with a blotter save about the hyphae and studied in a hanging drop over a damp chamber. In all experiments the damp chamber (made from a piece of thick cardboard) was placed in a Stender dish, the bottom of which was covered with the same solution as used in the hanging drop. I found this to be of the greatest importance for if there was any considerable variation in the concentration of the hanging drop and the fluid of the damp chamber a very considerable change would result in the concentration of the hanging drop producing very material alterations in the rate of growth and turgor. In this way the damp

chamber was also constantly kept saturated and the drying up of the drop prevented. Having selected from the drop culture a plant for testing, the cover glass was quickly freed from all fluid possible by means of the feathered edge of blotting paper, washed in the solution to which it is to be transferred, placed in a Stender dish and covered with the fluid after the manner described for germinating the spores. This insured an immediate action of the substratum and, owing to the relatively large volume of the substratum, it is not subject to any material change through the metabolism of the plant or the diffusion of any portion of the previously used culture fluid which might still cling to the cover glass through the capillarity of the paper or mycelia. Of course when the period of recovery from a change was short it was only necessary to wash the culture in the fluid to which it was to be transferred and mount it on the damp chamber in a fresh drop of this fluid. Knowing, by experiment, approximately when the plants would recover from the change of substratum, the cover glass was wiped dry, leaving a hanging drop on the mycelia, and placed on the damp chamber.

It was possible to determine the exact moment of renewal of growth after a change of substratum owing to the fact that this was announced by the enlargement of the apices of the hyphae, which were very constant and characteristic features. The turgidity of the plant was tested at this moment, although the swelling would go on for varying lengths of time, amounting in some cases to 10 or 15 minutes before a branch or usually branches would appear that increased the length of the hyphae.

Every precaution possible was exercised to secure accurate measurements of the turgidity. With the first sign of the enlargement of the apex the cover glass was freed of the fluid and immersed in the plasmolyzing fluid, isotonic solutions of sodium nitrate being used. A stock of the various percentages required was made up and used through all the experiments, and fresh volumes were taken from these jars for each experiment. The turgor force of a plant recovering from a change was always compared at the same time with the turgor force of a plant that had germinated and grown in the same strength of fluid to which the trial plant had been changed. In this way two plants in the same condition of development were compared, the one with the turgor

normal to the solution in which it was growing and the other had so adjusted its turgor to this same concentration of the substratum that growth became possible. It was found, after trying a certain percentage of the plasmolyzing solution on a plant without producing plasmolysis that any subsequent tests with higher strengths were unreliable, giving too high values to the turgor force. This is doubtless due to the fact that a solution too weak to plasmolyze does cause a concentration of the cell contents as is seen from the reduced size of the hyphae. One of the surprising features of the work was the variability of the turgidity, so that it was not at all safe to rely upon any determination of the turgor force of plants grown in a given concentration as a basis for comparison of plants changed to this concentration; as mentioned above, a test plant was always used as a check upon all measurements. This variability of the turgidity was also observed while working with Prof. Pfeffer, for whose courtesy in extending to me the privileges of the laboratory, I wish to return thanks.

Some of the results of the experiments upon *Mucor* will be found in the first table. The first column shows the average temperature of the day on which the experiment was made. In the second column is the record of the changes of the substratum; thus 0–4 indicates that the spores were grown in a simple nutrient solution and transferred to a nutrient solution containing 4% KNO_3. The third column shows the time elapsing after the change before growth appeared. For example, in the first experiment the culture was transferred at 8.10 to a 4% KNO_3 nutrient solution. Growth ceased till 10.13, or growth was renewed after two hours and three minutes. The turgidity in percentages of $NaNO_3$ is found in the fourth column. A — after a number indicates that this percentage did not plasmolyze as perfectly as in the case of the plant used as a check and for comparison. A + signifies the reverse condition, and that the plasmolysis was more severe than in the check plant. N. P. indicates that the percentage used failed to plasmolyze at all the hypha. The last column includes the measurements of the turgidity of the plants used as checks and for comparison with the plant subject to the change of substratum. Considering the second experiment, 18 in the fifth column indicates that this was the turgor force of plants germin-

ated and grown in a 4% KNO_3 nourishing solution on the day of the experiment, while in the fourth column 18 — records the fact that this same strength of the plasmolyzing solution did not plasmolyze as strongly the plant that was subjected to the change of concentration.

I.

1	2	3	4	5
21.	0–4	2.3	17 n. p.	18
21 5	"	2.15	18 —	18
22.	"	1.43	18	18
21.5	"	1.35	18 —	18
22.	"	1.45	18 +	18
22.6	"	.59	20 +	20
22.6	"	1.33	20 +	20
22.6	"	1.25	20 +	20
21.5	"	1.50	18 —	18
20.8	"	.45	18	18

These are a few illustrations taken from many experiments to give an idea of the range of variation in different cases. The interesting feature appears in columns 4 and 5. All the measurements show practically the same result, that before growth is renewed the turgor force has become equal to the turgor of plants growing normally in this solution. Scores of experiments were performed testing the plants as in the first experiment with a lower

II.

1	2	3	4	5
22.	4–0	15 min.	8	8
22.6	"	40	8	8
22.6	"	34	8	8
21.5	"	20	8 —	8
21.5	"	50	8 —	8
25.	"	35	7 n. p.	8
25.	"	30	8	8
23.	"	47	8 —	8
22 5	"	28	8 +	8
21.	"	32	8 —	8

percentage than the check plant indicated. The result was either as in this case a lack of plasmolysis or only slight indications of it.

The three measurements indicating a turgor force of 20 are introduced to illustrate the variations that appear without any

assignable reason. From other experiments it is not at all possible to conclude that temperature is the cause of the rise.

In Table II. will be found some of the results obtained by germinating spores of *Mucor* in a nourishing solution containing 4% KNO_3 and transfering to a simple nourishing solution.

Here again there is a complete agreement between the turgor forces of the plants grown under the different conditions; the slight variations indicated by the — or the + are of little importance as they are found to be equally distributed throughout my experiments. The turgor force was not always as constant as indicated in column 5, some tests showing a force of 7 and a few of 9. In the majority of cases it was very constant.

In Table III. are given a few of the results of experiments on *Botrytis*.

III.

1	2	3	4	5
21.	0-4	.41 min.	22	22
22.2	"	.60	22 —	22
22.6	"	1.12	22 n. p.	22
21.	"	1.10	22	22
21.8	"	.50	23	23
23.4	"	1.35	22 n. p.	23
24.	"	1.53	21	21

IV.

1	2	3	4	5
22.	4-0	50 min.	12 n. p.	13
22.8	"	51	12	12
21.6	"	55	12 +	12
23.4	"	42	12	12
22.6	"	62	12 —	12
22.6	"	38	12	12
24.	"	46	12	12

The generally longer period of recovery noted in Table IV. is not due apparently to the considerably higher turgor force developed in the plants, since the difference of turgor in the 4% and null solutions is practically the same in both *Botrytis* and *Mucor*, *i. e.*, 10. On the other hand the periods of recovery found in Table III. are somewhat shorter than in Table I.

The tests upon *Penicillium* were more unsatisfactory than any of the others owing to the fact that the turgor varied considerably

on different occasions. It might naturally be supposed that this was due to some faulty application of the plasmolyzing solutions. More tests, however, were made upon *Penicillium* than upon any of the others in the hope of securing uniform measurements. But the fact remained that there would occur variations of one to three per cent. But this fluctuation does not affect the results of the work because of the practice of comparing every measurement obtained in an experiment with the turgor force of a test plant.

V.

1	2	3	4	5
21.	0–4	63 min.	16 —	16
21.4	"	41	16	16
21.8	"	76	15	15
21.8	"	70	13	13
21.6	"	46	16	16
22.	"	47	16 —	16
21.4	"	88	16 +	16

VI.

1	2	3	4	5
22.6	4–0	21	10 —	10
22.8	"	25	10	10
22.8	"	45	10	10
22.	"	38	8 +	8
21.4	"	22	9 +	9
21.6	"	50	9	9
21.	"	42	9	9

It is very noticeable that the turgor force has a marked influence in regulating the period of recovery. While in Table III. it is more usually over one hour and averages somewhat higher than the table indicates, in Table V. the recovery is decidedly shorter. The same ratio exists in Tables IV. and VI. In the case of *Botrytis* there was produced by the changes of the substratum a difference of 10 in the turgor force while in *Penicillium* this only amounted to about 6. The results obtained in the experiments with *Mucor* are not so easily explained, for while the period of recovery is shorter than in *Penicillium*, when changing from 4 to 0, the reverse change results in a rather longer period of retardation approximating that of *Botrytis*. This latter relation would be be expected since the difference in turgor produced by the two

substrata are about equal, *i. e.*, 10. It is quite possible that the structure of the two plants has much to do with the variation, the one being essentially weak in this particular and the other septate.

I was interested to substitute NaCl for KNO_3 for the purpose of determining whether the replacement of a salt that is of so vital importance to the plant with one of slight value would produce any pronounced results. The action of this salt was most striking and it was found impossible to use only comparatively weak solutions. *Mucor* would not tolerate as well a 2 per cent. solution as the corresponding value of KNO_3, and this often produced bursting in *Botrytis*. *Penicillium* was more resistant and would usually withstand a change from 4 to 0. The results may be summarized as follows:

Mucor in a temperature averaging 24.5 recovered very slowly in changing from a nutrient solution to 2% NaCl solution. The period ranged from 3 hours and 40 minutes to 5 hours and 46 minutes and the turgor was found to be 21–22 in the recovering and test plant. In transferring from 2 to 0 recovery was effected in 19 to 43 minutes, turgor force 9. This higher turgor force (9) was probably due to the temperature and this is to be noticed generally in all work done in the summer. *Penicillium* recovered, temperature 25, from a change of 0 to 2 in 1.24 to 2.36 hours, turgor in all comparative tests alike and ranging from 19 to 20. Transferring from 2 to 0, recovering in 36 to 56 minutes, turgor ranging from 11 to 12. Changed from 0 to 4 solution, recovery 1.55 to 2.50 hours, turgor 29–30; transferred from a 4 to 0, recovery in 31 to 70 minutes with turgor force as stated above. *Botrytis*, transferred from 0 to 2% solution, recovered in 1.40 to 3.10 hours, turgor 23–24. Changing from 2 to 0, recovering in 30 to 80 minutes, turgor 13. These tests show the same results and general relations as those found in Tables I and II. The excessive turgor pressure is manifestly due to the injurious and retarding influence of the NaCl upon the rate of growth.

A summary of the results obtained with the higher concentrations of the KNO_3 nourishing solutions is given below. *Penicillium* was found to have the greatest power of adaptation and would sometimes withstand a transfer from 19 to 0 solution without seri-

ously bursting all of the hyphae. In transferring *Penicillium* from 0 to 9 recovery followed in 2 to 5.30 hours, turgor 28. Changing from 9 to 0, recovery in 50 minutes to 1.50 hours, turgor 11; changed from 0 to 14, recovery 2.40 to 7 hours, turgor 36; changed from 14 to 0 recovery, 55 to 1.41, turgor 11; changed from 0 to 19, recovery 5.40 to 12.18 hours, turgor 43. *Botrytis* 0 to 9, recovery in 1.56 to 6.10 hours, turgor 34, 9 to 0, recovery 48 to 2.21, turgor 13, 0 to 14, recovery in 3.10 to 7.43, turgor 39. Less importance is attached to these experiments owing to the frequent bursting of the hyphae. However, they show that the same relations exist between the turgor of the recovering plant and the test plant as appeared in the preceding work.

The result of the work was unexpected and seems to indicate that a much closer relation exists between turgor and growth in these simple plants at least than has been supposed. I had anticipated finding several percentages of difference between the turgor force of the plant just recovering from a change of substratum and that of a plant germinated and grown in this substratum. It appears from these experiments that there is a necessity of a certain turgor force before growth is possible and that growth cannot occur until a turgor pressure has been reached which is normal to the plant growing in the given solution. That turgor is not alone the cause of growth is apparent from the acceleration of the rate of growth after a change of substratum. In the following statements it will be seen that there is always a short period of slow growth. The figures represent divisions on the micrometer eye piece, the periods of measurement being 5 minutes.

1 5	1.5	1.	2.	.5	.5
1.	1.5	2.	2.5	1.	.5
2.5	2.5	3.5	3.	.5	2.
3.	3.	4.5	3.	1.5	2.5
3.	3.5	6.	3.	3.	3.

Again it is to be noticed that when the turgor pressure was enormously increased by changing from high to low concentrations no growth followed but the quite uniform period of recovery indicates that some agency other than the widely various turgor pressure was at work and it seems quite probable, as has been suggested by Townsend and by True, that the cessation of growth is to

be considered as the response of the irritability of the protoplasm due to shock. In fact, the measurements obtained by Townsend by injuring the hyphae and observing the period of recovery correspond with some of the above measurements. This shock also entered as a factor in the changes from low to higher concentrations, but the periods of recovery constantly grew longer in as much as the accumulation of turgor force is a slow process and requires more time than recovery from shock. In the cases where we are dealing with a reduction of the turgor force a comparative short period, *i. e.*, the recovery from shock, is sufficient to adjust the turgor since this is brought about by purely physical laws. The accumulation of high pressures in the plants retarded in their growth by NaCl and the low turgor of the plants growing rapidly in solutions of slightly lower concentration, a phenomenon that has often been discussed, seem to point to the same conclusion. A certain maximum turgor force is necessary to start growth. If for any reason growth is checked there follows an increase of the pressure tending to continue the normal growth. Under the reverse conditions a rapid growth lowers the pressure which is, however, high enough to play its rôle in growth. Eschenhagen has demonstrated that the turgor pressure of fungi is considerably in excess of the substratum in which they are growing and this is to be looked upon as an adaptive provision permitting changes of substratum. It is possible that there must also be a certain excess of turgor to inaugurate growth and higher than is actually required to sustain it. Naturally a rapid growth expends a portion of this surplus reducing turgor. These variations of pressure are comparable to the work done in overcoming the inertia of an object at rest, a greater force is required to start the motion that is necessary to maintain it. A better comparison is found in a water reservoir with a constant supply and a siphon discharge. A certain maximum volume of water is required to bring the siphon into action, but once in action it will continue the flow of water, though the volume in the reservoir is greatly reduced. Furthermore, by reducing the caliber of the siphon the volume rises, but by increasing it the volume diminishes in both cases in proportion to the area of the siphon.

Turgor then is regulatory in its action. We can neither say

that it controls growth nor, on the other hand, is it controlled by growth, but it must sustain a certain fixed relation and come into harmony with the other existing forces and conditions before growth is possible.

The more important features of the work may be summarized as follows:

1. The hyphae of fungi possess remarkable powers of adaptation but show considerable individuality in this respect.

2. The turgidity varies under uniform conditions.

3. The moment of recovery from a change of concentration can be accurately noted since it is indicated by an apical enlargement preceding the elongation of the hypha.

4. Changes from a low to a higher conception of the substratum resulted in a steadily increasing period of recovery in proportion to the concentration of the substratum.

5. Recovery from a change from a higher to a lower concentration was controlled only to a limited extent by the degree of concentration. But there was also to be observed an individuality peculiar to the genus employed which brought about these changes in shorter or longer periods of time.

6. The turgidity of a plant recovering from a change of concentration is the same as that of a plant germinated and growing in the concentration to which the trial plant has been changed.

7. Turgidity appears to be a regulatory force.